

Molecular Characterization of Hospital- and Community-Acquired *Streptococcus agalactiae* Isolates among Nonpregnant Adults in Isfahan, Iran

Abstract

Background: The increasing incidence of Group B Streptococcus (GBS) infection among nonpregnant adults has become of growing clinical and public health concern. The current study investigated the distribution of important virulence determinants and antibiotic susceptibility of GBS isolates causing community acquired (CA) and hospital acquired (HA) infections among nonpregnant adults. **Materials and Methods:** A total of 62 GBS, including 31 CA GBS and 31 HA GBS, were collected from a teaching hospital in Isfahan, Iran. Capsular polysaccharide genotypes (CPS), PI 1, PI 2a, PI 2b, and hypervirulent GBS adhesin (hvgA) virulence genes and antibiotic resistance profiling were determined. **Results:** There were 19 (30.6%) cases of underlying disease that diabetes mellitus (20.9%) was most common. The rate of multidrug resistant GBS strains was accounted for 29%. Distribution of macrolide resistant phenotypes was as follows: constitutive macrolides, lincosamides, and streptogramin B (MLSB) (15 isolates); inducible resistance to MLSB; and L phenotype (each 5 isolates) and M phenotype (1 isolate). V and Ia serotypes were the most predominant capsular type in HA GBS and CA GBS isolates, respectively. The most frequent pilus types were PI 1, PI 1+PI 2a, PI 1+PI 2b, and PI 2a. PI 1 and PI 1+PI 2a had significantly different distributions between CA and HA GBS isolates. Three CA GBS isolates (9.6%) were positive for hvgA gene that belonged to clonal complex 17/sequence type 17/CPS III/PI 1+PI 2b lineage. **Conclusion:** There was a significant difference in the distribution of PIs among CA GBS and HA GBS isolates in our region.

Keywords: Iran, microbial sensitivity tests, streptococcus agalactiae, virulence factors

Introduction

Group B *Streptococcus* (GBS) is an opportunistic pathogen causing infections in pregnant women, newborns, and the elderly. GBS colonizes in the genital and gastrointestinal tracts in both men and women.^[1] In recent decades, reports of serious GBS infections in adults have been increased.^[2] Some underlying medical conditions are associated with GBS infection in nonpregnant adults such as diabetes mellitus, heart disease, renal dysfunction, and malignancy.^[3] Most infections caused by GBS are often community-acquired (CA), but also hospital-acquired (HA) infections occur in the health-care setting.^[4] Surgical-site infection especially following cesarean section, urinary tract infection (UTI), and joints and soft-tissue

infection are the most clinical manifestation of GBS infections among hospitalized patients.^[5]

GBS has a wide variety of virulence factors that facilitate its ability to cause infection, such as polysaccharide capsule, surface antigenic proteins, and pilus islands involved in the adhesion; invasion of host cells; and evasion from the immune system.^[6] Three types of pilus islands including PI-1, PI-2a, and PI-2b were identified in GBS.^[7] PI-1 plays an important role in the evasion from the innate immune system and macrophage-mediated killing. PI-2a has been shown to be more important for adherence to host cells and biofilm formation. PI-2b was suggested to increase intracellular survival in macrophages and invasiveness of GBS.^[8] Because pili play an

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important role in GBS colonization, invasion, and disease progression, the type of pilus likely impacts the potential invasiveness of GBS.^[9]

Another important virulence factor in GBS is hypervirulent GBS adhesin (HvgA). HvgA is a ST-17 (sequence type)-specific surface-anchored protein. The role of HvgA protein in the progression of meningitis infection due to its ability to cross the intestinal and blood–brain barriers has been confirmed. HvgA appears to be a promising target for vaccine and development of antibacterial strategies.^[10]

There are a few data about molecular characteristics and virulence factors of HA-GBS and CA-GBS strains in our country. The aim of the current study was to identify and compare capsular serotypes, *hvgA*, and pilus island genes and antibiotic susceptibility profiling of HA-GBS and CA-GBS collected from a teaching hospital in Isfahan, Iran.

Materials and Methods

Bacterial strains

CA-GBS was defined as an isolate that was obtained either from an outpatient or inpatient ≤ 48 h after hospital admission. HA-GBS was defined as an isolate that was obtained from an inpatient >48 h after hospital admission. In cases of infection symptoms, the definition of infection followed the guidelines published by the Centers for Disease Control and Prevention.^[11] Samples from pregnant women or containing two or more bacterial species were excluded from the study. A total of 62 GBS (31 HA-GBS and 31 CA-GBS) were collected from the hospital teaching laboratory from June 2016 to December 2018. The isolates included HA and CA-GBS recovered from noninvasive infections associated with UTI, vaginitis, tracheal tube secretions, and abscess ($n = 46$) and CA-GBS isolates from urine samples of asymptomatic nonpregnant women ($n = 16$). All of the GBS isolates were identified based on typical morphology of colonies on blood agar, beta-hemolytic activity, Gram stain, catalase test, CAMP reaction, and amplification and detection of 952 bp *dltS* gene, specific for GBS encoding specific histidine kinase by polymerase chain reaction (PCR).^[12]

Antibiotic susceptibility

The disc diffusion method was used to determine the susceptibility pattern of the nine antibiotics listed in Table 1. The Clinical and Laboratory Standards Institute 2016 edition criteria were used to classify the isolates as susceptible, intermediate, or resistant.^[13] Multidrug resistance (MDR) was defined as the resistance to any three or more antimicrobial agents of different classes tested in this study. Detection of inducible clindamycin resistance was performed using D-zone test method as previously described,^[13] and inducible resistance to macrolides, lincosamides, and streptogramin B (iMLSB

with blunting of a zone of inhibition of clindamycin); constitutive MLSB (cMLSB with resistance to clindamycin and erythromycin); M phenotype (resistant to erythromycin only but not clindamycin by efflux mechanism); and L phenotype (resistant to clindamycin only without D shape) were identified.

DNA extraction

All strains were grown on Trypticase Soy Agar (Merck, Germany) supplemented with 5% sheep blood and incubated for 24 h at 37°C. The DNA of GBS isolates was extracted using phenol-chloroform method with some modification. Briefly, a loopful of bacterial biomass was suspended in 300 μ l of TSE buffer (50 mM Tris hydrochloride [pH 7.5], 25 mM ethylenediaminetetraacetic acid, 10% SDS), and the suspension was heated at 95°C for 20 min. Then, equal volumes of the phenol: chloroform (pH 8) were mixed with the suspension and centrifuged at 9000 \times g for 5 min. The supernatant was introduced into the new microtube, and 300 μ l of chloroform was added and centrifuged at 9000 \times g for 5 min. The extracted DNA was precipitated with cold absolute ethanol. DNA was washed with 70% ethanol and resuspended in TE buffer and stored at -20°C for molecular assay.

Capsular genotyping

Capsular genotyping was performed using nine pairs of primers as described by Poyart *et al.*^[14] The two sets of multiplex PCR program were carried out by the first denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min; annealing at 58°C, for 1 min; and an extension at 72°C for 1 min. The final extension was done at 72°C for 5 min. A primer pair (*dltS*-F and *dltS*-R) targeting the GBS-specific *dltS* gene was also included as an internal positive control and a microtube containing PCR reagents with distilled water instead of the DNA template was used as the negative control.^[12] In addition, *Streptococcus agalactiae* ATCC 13813 was used as the positive control.

Pilus island distribution

Pilus island genotyping was performed using primer pairs and multiplex PCR reactions as previously described. In addition, the lack of PI-1 genes was confirmed by a set of primers that amplify the regions flanking the PI operon.^[15] The PCR products were analyzed by electrophoresis in a 2% (wt/vol) agarose gel [Figure 1].

Detection of hypervirulent GBS adhesin gene

For the detection of *hvgA* gene, a 210-bp region was amplified by ST-17S and ST-17AS primer pairs as previously described.^[16] This genetic region has been described as being present in GBS strains belonging to the CC17/ST-17 clone as hypervirulent lineage.

Table 1: Antibiotic susceptibility profile of community-acquired isolates - Group b streptococci and hospital-acquired isolates - Group b streptococci

Antibiotic	CA-GBS, n (%)			HA-GBS, n (%)		
	S	I	R	S	I	R
Penicillin	31 (100)	-	-	31 (100)	-	-
Cefepime	31 (100)	-	-	31 (100)	-	-
Ceftriaxone	31 (100)	-	-	31 (100)	-	-
Cefotaxime	31 (100)	-	-	28 (90.3)	-	1 (3.2)
Vancomycin	31 (100)	-	-	31 (100)	-	-
Tetracycline	2 (6.4)	-	29 (93.5)	3 (9.6)	-	28 (90.3)
Levofloxacin	26 (83.8)	2 (6.4)	3 (9.6)	24 (77.4)	2 (6.4)	5 (16.1)
Clindamycin	12 (38.7)	8 (25.8)	11 (35.4)	16 (51.6)	6 (19.3)	9 (29)
Erythromycin	15 (48.3)	5 (16.1)	11 (35.4)	11 (35.4)	10 (32.2)	10 (32.2)
iMLSB		2 (6.4)			3 (9.6)	
c MLSB		8 (25.8)			7 (22.5)	
M phenotype		1 (3.2)			-	
L phenotype		3 (9.6)			2 (6.4)	
MDR		9 (29)			9 (29)	
Total		31			31	

MLSB: Macrolides, lincosamides, and streptogramin B, iMLSB: Inducible resistance to macrolides, lincosamides, and streptogramin B, S: Susceptible, I: Intermediate, R: Resistant, MDR: Multidrug resistance, GBS: Group B streptococci, CA: Community acquired, HA: Hospital acquired

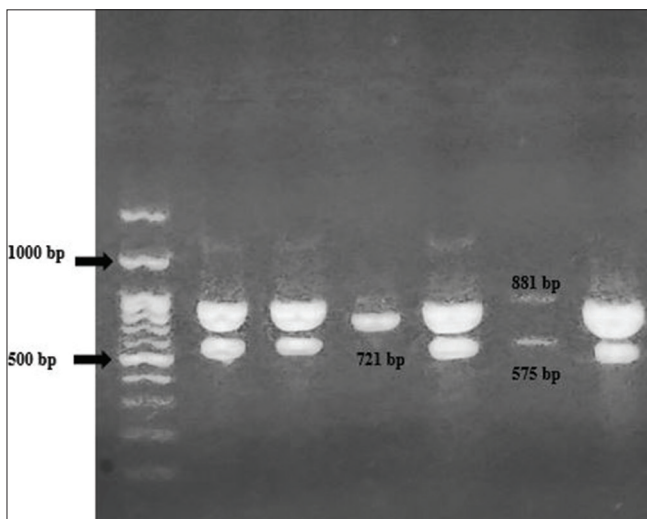


Figure 1: Agarose gel electrophoresis of the multiplex polymerase chain reaction amplification of pilus island genes. 100 bp DNA ladder (Fermentas), PI-1 gene: 881 bp, PI-2a gene: 575 bp, PI-2b gene: 721 bp

Statistical analysis

To assess if any pilus islands and capsular type is associated with CA-GBS or HA-GBS isolates, the distribution of each of Pilus island and capsular serotype was examined against random distribution using the Pearson's Chi-square test. The association between pilus island and capsular serotype distributions was assessed using Fisher's exact test. Differences were considered statistically significant at $P < 0.05$.

Results

Demographic data and clinical characteristics

A total of 53 (85.4%) GBS isolates were recovered from female patients. Among CA-GBS isolates, 28/31 patients

and, in HA-GBS, 25/31 patients were female. The median age for patients with HA-GBS and CA-GBS infections was 68 and 40 years, respectively. A total of 19 patients had an underlying disease in that diabetes mellitus (13 patients) was the most common underlying condition for GBS acquisition followed by heart disease and renal dysfunction (3 and 2 patients, respectively) and malignancy (1 patient). The overall underlying disease among patients with CA-GBS infection was greater than HA-GBS (12 patients vs. 7 patients). UTI was the most prevalent infection among the patients (49/62, 79%) followed by vaginitis (8/62, 12.9%), tracheal tube secretions (4/62, 6.4%), and abscess (1/62, 1.6%). More data are presented in Table 2.

Antibiotic susceptibility

The results of antibiotic susceptibility pattern with the Kirby-Bauer method revealed that all of the GBS strains were Susceptible to penicillin, vancomycin, cefepime, and ceftriaxone. Multidrug-resistant GBS strains were detected in CA-GBS and HA-GBS groups (29%). Results of the D-zone test showed that most GBS strains showed cMLSB phenotype in association with erythromycin resistance. More details are shown in Table 1.

Capsular genotyping and Pilus island distribution

Molecular capsular typing of the capsular polysaccharide serotypes (CPS) gene showed the most prevalent serotype among all GBS to be Ia (22.5%) followed by III and V (each 20.9%), Ib (17.7%), II (9.6%), IV (8.06%), and VI (1.6%). Comparison of capsular serotype distribution between the two groups revealed that the prevalence of Ia, Ib, and II serotypes was higher in CA-GBS isolates. V and Ia serotypes were the most predominant capsular type in HA-GBS and CA-GBS, respectively [Figure 2]. The

Table 2: Distribution of 62 noninvasive community-acquired isolates - Group B streptococci and hospital-acquired isolates - Group B streptococci isolates among patients

Sample	Number of isolates	Patient condition (n)	Sex (n)
Urine	49		
Catheter-associated UTI	7 (HA-GBS)	Renal dysfunction (1)	Female (6), male (1)
UTI	26 (7 CA-GBS, 19 HA-GBS)	Diabetes mellitus (10), renal dysfunction (1)	Male (5), female (21)
Asymptomatic	16 (CA-GBS)	Heart disease (1), Diabetes mellitus (3)	Female
Tracheal tube secretions	4 (HA-GBS)	Heart disease (2)	Male (2), female (2)
Vaginal discharge	8 (CA-GBS)	Vaginitis (8)	Female
Abscess	1 (HA-GBS)	Malignancy (1)	Male

GBS: Group B streptococci, CA: Community acquired, HA: Hospital acquired, UTI: Urinary tract infection

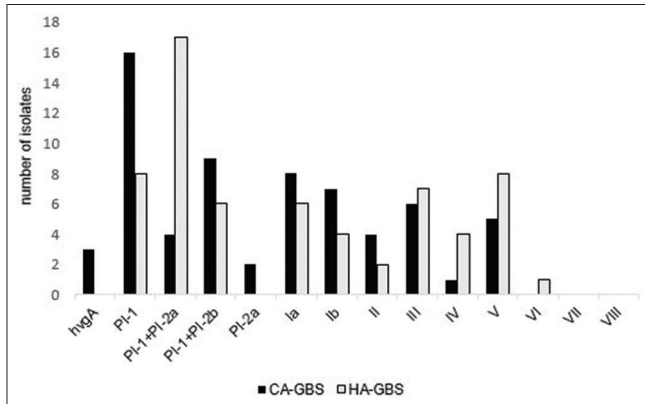


Figure 2: Distribution of capsular genotypes, pilus islands, and hvgA gene among community-acquired Group B streptococci and hospital-acquired Group B streptococci

dominant molecular profile among the GBS isolates was CPS III/PI-1/tetracycline^R.

The results showed that each GBS isolate expressed at least one pilus type. In general, PI-1 was the most common type (24/62, 38.7%) followed by PI-1+PI-2a (21/62, 33.8%), PI-1+PI-2b (14/62, 24.1%), and PI-2a (2/62, 3.2%). PI-1 and PI-1+PI-2a had statistically significant different distributions between CA-and HA-GBS isolates ($P < 0.03$ and $P < 0.001$, respectively).

Hypervirulent GBS adhesin gene distribution

Only three isolates of CA-GBS were positive for the *hvgA* gene. These isolates belonged to clonal complex 17/sequence Type 17/CPS type III/PI-1 + PI-2b lineage and were recovered from two women and one man with UTI.

Discussion

Recently, the number of infections caused by *S. agalactiae* in nonpregnant adults is increasing; the majority of patients had underlying conditions such as diabetes mellitus, malignancy, genitourinary abnormalities, neurologic deficits, cirrhosis, renal dysfunction, steroid uses, heart disease, and AIDS.^[17-19] In the current study, 30.6% of patients had a medical underlying condition in that diabetes mellitus, heart disease, renal dysfunction, and malignancy were observed. The rate of the underlying condition in

patients with CA-GBS infection was higher than that of the HA-GBS group. All of our patients with diabetes mellitus had a positive urine culture. In the other studies, diabetes, chronic renal diseases, and malignancy were the most prevailing conditions for GBS disease.^[20,21]

Although GBS disease can occur in adults of all ages, the median age is over 60 years.^[22] Inpatients of this study had a median age of over 60 years, which confirmed the role of age as a risk factor for the acquisition of GBS infection in adults.

The antibiotic susceptibility pattern of our isolates showed that in our region the rate of resistance to most antibiotics was low except for tetracycline. However, on the other hand, the emergence of MDR strains should be noticed. Erythromycin, clindamycin, and levofloxacin are alternative therapeutic agents for β -lactam allergic patients. However, some of our GBS isolates have been recognized with resistance to all of them in addition to tetracycline. The resistance to erythromycin and clindamycin in our study was 33.8% and 32.2%, respectively. In previous studies, in various regions of our country, resistance rate to erythromycin was reported to be 35%,^[23,24] 52%,^[25] and 100%.^[26] Resistance rate in other parts of the world was different. In Ethiopia, there was a resistance rate of 26% and 21% to erythromycin and clindamycin, respectively.^[27] However, research in China documented that resistance to erythromycin and clindamycin was 46% and 29%, respectively.^[28] Current research revealed that most of our erythromycin-resistant GBS isolates showed cMLS_B phenotype. Many studies also revealed such results; for example in Iran,^[23] Serbia,^[29] Ethiopia,^[27] and Egypt.^[30] In many studies, the association between serotype and certain antibiotic resistance has been reported. We did not detect any statistical association, but most of our erythromycin-resistant GBS isolates belonged to CPS type Ia. In many investigations, the resistance rate of GBS isolates to levofloxacin was reported low, such as Italy and Taiwan accounted for 2.9% and 6.2%, respectively.^[31,32] The resistance rate of our GBS isolates to levofloxacin was higher (12.9%) and similar to Argentina.^[33]

The prevalence and distribution of GBS serotypes are geographically distinct.^[34] A little surveillance of other

studies in various regions of Iran illustrated the similar distribution of the most prevalent serotypes (Ia, Ib, II, III, and V), however, in comparison to our study, in previous reports, IV serotype less has been reported.^[23,35-37] In addition, previous reports from our country revealed that III serotype was the most frequent capsular type among human-derived GBS isolates.^[23,25,37] A study in China showed that III and Ia serotypes were the most predominant among HA-GBS and CA-GBS in adults.^[38] Clonal dissemination of serotype VI in central Taiwan and VI and VIII serotypes in Japan showed different distribution among adults.^[39,40] In Malaysia, serotype VI was found among isolates in adults with skin and soft-tissue infections.^[41] Serotype distribution of GBS isolates in the current study was similar to data from the United States. In the United States, Ia and V serotypes were dominant among GBS isolates in nonpregnant adults.^[42]

Pilus island distribution among HA-GBS and CA-GBS isolates of the current study showed statistically significant differences. Among the CA-GBS isolates, PI-1 had the highest prevalence followed by PI-1+PI-2b, PI-1+PI-2a, and PI-2a. However, in HA-GBS isolates, results were different and a high prevalence of PI-1+PI-2a ($P < 0.001$) was detected. Data on the distribution of pilus islands in our country were very low. Only one study in our country showed that PI-1+PI-2a was the most prevalent allelic form of pili among GBS isolates recovered from adults.^[35] Other investigations from Portugal and China documented that PI-2a was the most frequent pilus island.^[7,43] A combination of PI-1 and one of the PI-2 variants enhances the invasiveness of GBS strains.^[9] The high prevalence of PI-1+PI-2a among HA-GBS strains in this study may be due to this principle that a combination of PI-1+PI-2a could enhance survival of GBS stains by increasing colonization, resistance to host immune system, starvation, and disinfectant effects used in the hospital environment.^[44]

Conclusion

Significant differences in the distribution of pilus islands and capsular serotypes among HA-GBS and CA-GBS isolates and detection of resistance to levofloxacin and MDR strains establish remarkable features of GBS strains in the current study. Additional studies are needed to investigate the role of pili and other virulence factors involved in immune evasion, host–cell interactions, and successful environmental persistence mechanisms such as biofilm formation. These data can help to define better infection control programs such as vaccine development and preventive therapeutic targets against the GBS population in the community and health-care setting.

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Conflicts of interest

There are no conflicts of interest.

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